PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Eric WICKSTROM

Serial No: 10/688,821 Group Art Unit: 1633

Filed: 10-16-2003 Examiner: POPA, ILEANA

Att. Docket No.: W1133/20008 Confirmation No.: 2530

For: COMPOUNDS AND METHODS FOR DIAGNOSTIC IMAGING AND THERAPY

DECLARATION OF ERIC WICKSTROM UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Eric Wickstrom, Ph.D., a citizen of the United States, hereby declare and state:

1. The resume attached as Exhibit A accurately reflects my professional credentials.

2. I am a co-inventor of the subject matter described and claimed in the present application.

3. I have reviewed the application and its prosecution history including the Office Action of July 26, 2007.

4. I understand from my review of the Office Action that claims 1, 3, 4, 7-14, 16, 26-31, 34, 41-45, 48, 50, 52, 54-56, 69-73, 75, 80, 83, 85, 86, and 88-92 stand rejected under 35 U.S.C. §103(a) as allegedly being obvious over Tomalia et al. (US Patent No. 5,714,166), in view of both Meade et al. (US Patent No. 6,713,046) and Basu et al. (Basu et al., Bioconjugate Chemistry 1997, 8, 481-466).

5. According to the attorneys, finding obviousness requires that the prior art reference (or references when combined) must teach or suggest all the claim limitations and that there must be some suggestion of motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings.

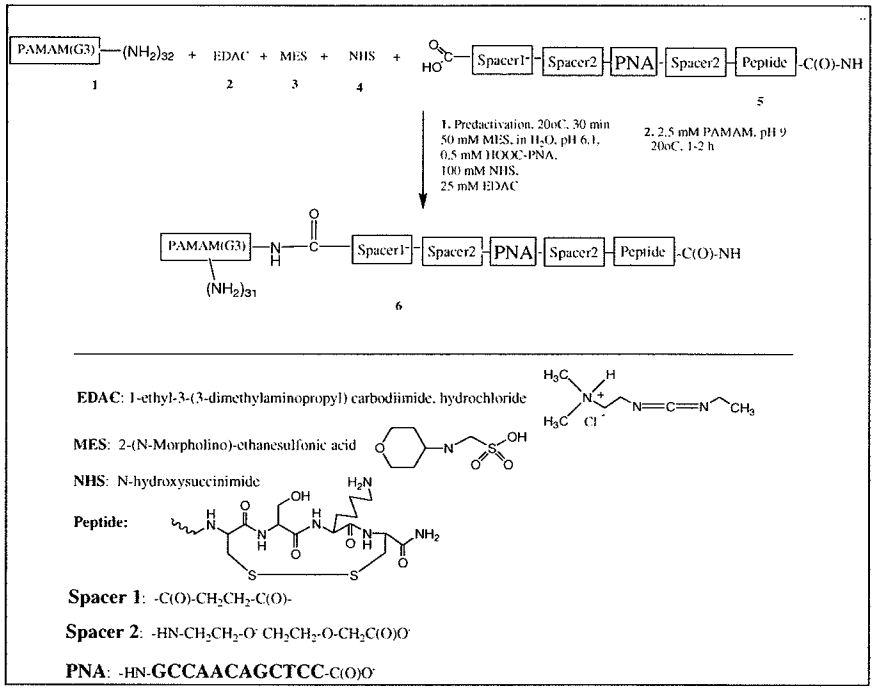
6. I am very familiar with Tomalia et al. (US Patent No. 5,714,166), Meade et al.

(US Patent No. 6,713,046) and the Basu et al. reference.

7. None of the cited references (alone or in combination) describes a compound X-L1-P-L2-T, wherein X represents a diagnostic or therapeutic agent, such as a radionuclide chelated to a dendrimer, P represents a PNA that can bind a nucleic acid, and T represents a cell surface target director, such as a moiety that can bind a cell-surface molecule, and wherein X, P and T are associated with identical or different spacers L1 and L2 to prevent steric hindrance and therefore lack teaching or suggesting all the claim limitations.

8. Attempted Synthesis of PAMAM-spacer-PNA-spacer-peptide. Early in my studies, I attempted to utilize the PAMAM dendrimer designed by Donald Tomalia, despite his stipulation that genetic material was never covalently bonded to PAMAM. Under my direction, members of my laboratory tried the following representative coupling protocol, with no yield.

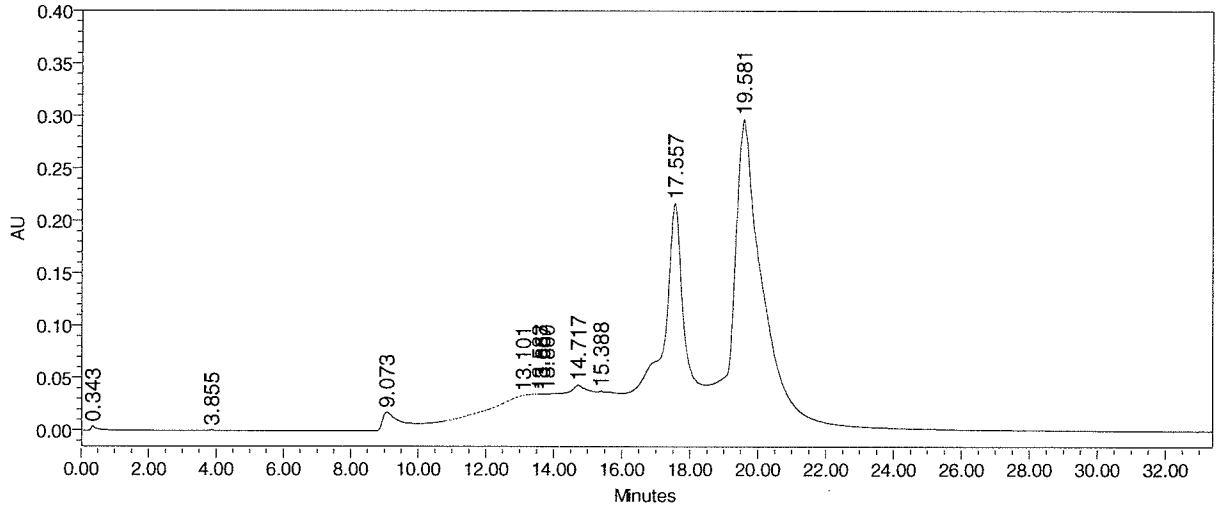
9. Synthesis of PAMAM-spacer-PNA-spacer-peptide: PAMAM(3G)-Sp_1-Sp_2-PNA-Sp_2-peptide.



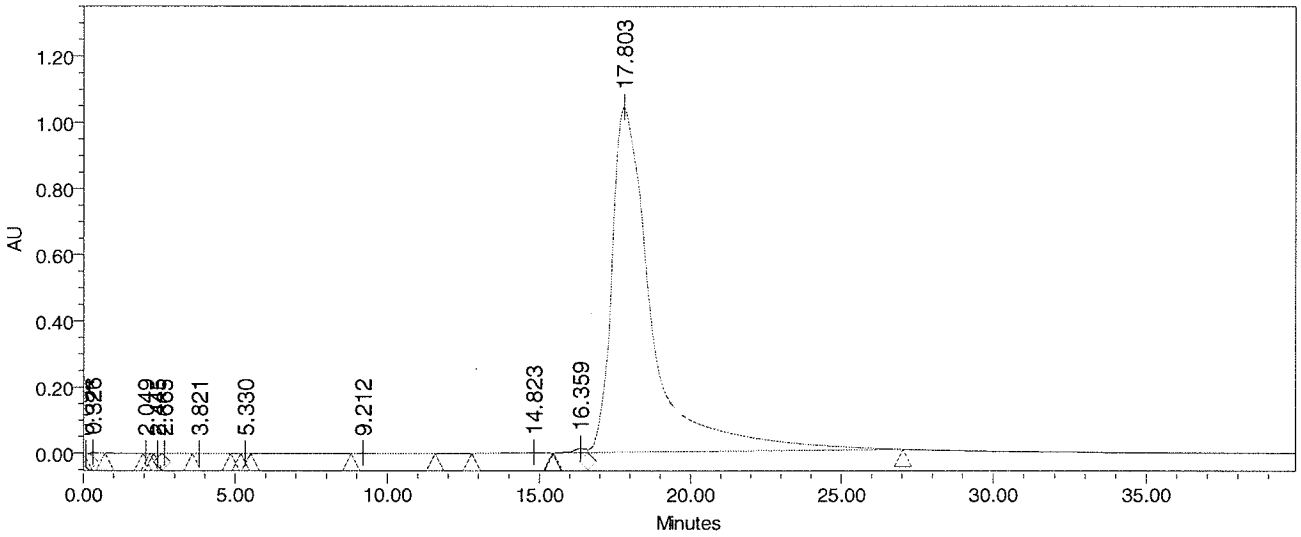
Reaction mixture:

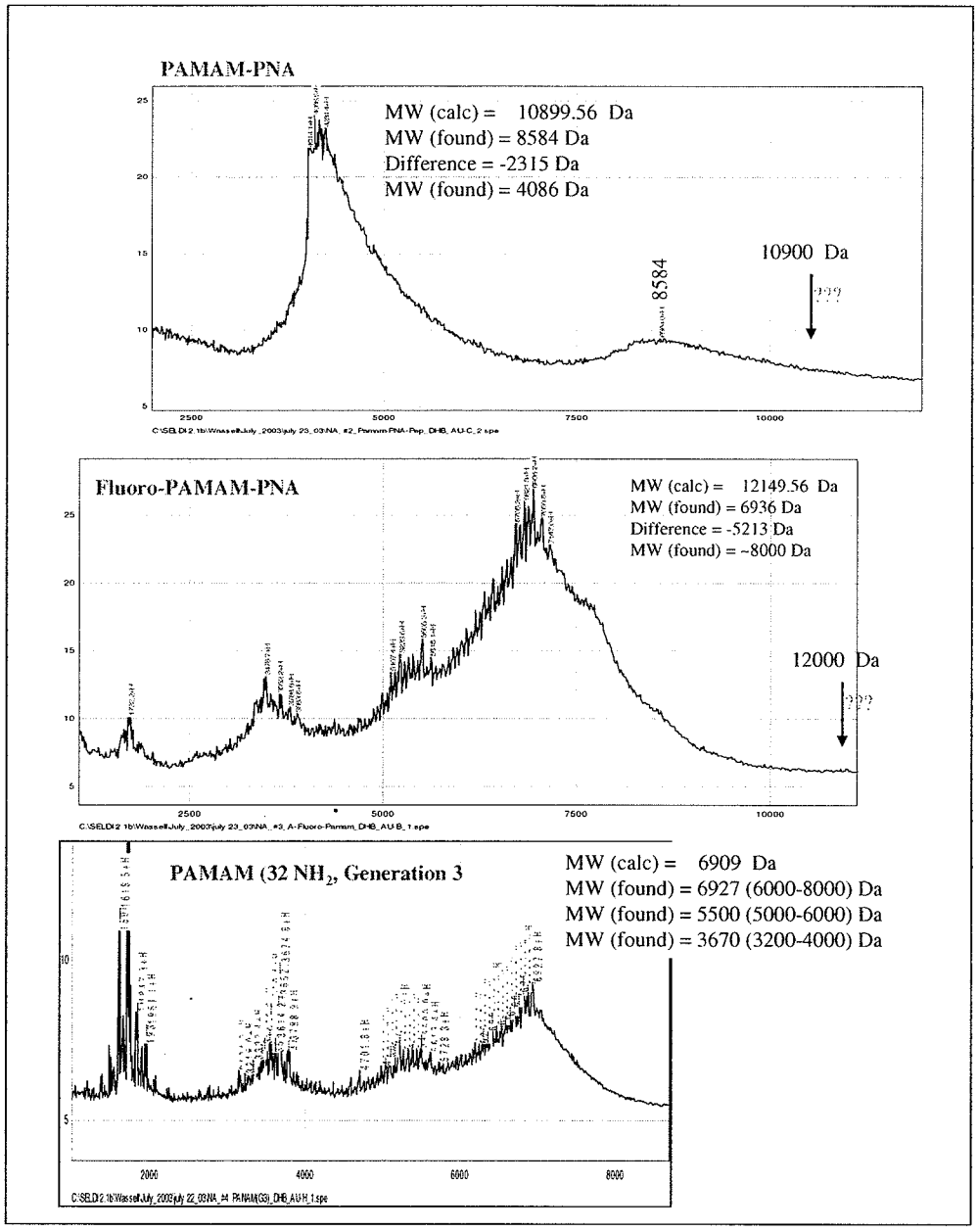
10.8 µL HOOC-spacer-PNA-spacer-peptide (100 A_{260}U/µL, 1.08 A_{260}U, 0.01 µmol)
2 µL MES (morpholinoethanesulfonate) buffer (0.5 M, pH 6.1)
2 µL 1 M NHS (N-hydroxysuccinimide) (2 µmol)
1 mg EDAC-HCl (water soluble carbodiimide) (5 µmol)
16 µL total volume
↓ 30 min activation, pH 6.1, 22°C.
2 µL PAMAM (3G) (20% w/w in MeOH, 0.05 µmol)
4 µL 0.5 M NaOH
22 µL total volume, pH 9
↓ 24 h
Size-Exclusion HPLC G2000 SW (part 08799)
↓
MALDI-TOF MS analysis (see exp N26)
HPLC analysis

10. Result: Sample Name: N-24-SE-3-gr-PAM-PNA_20h. Size Exclusion HPLC. PAMAM-spacer-PNA-spacer-peptide, Reaction mixture, 20 h (10 µl, 0.5 A_{260}U, in 50 µL of buffer D, monitored at λ=260 nm). Isocratic D/80 min, D=50% CH_3CN, 0.1% CF_3CO_2H in H_2O, T = 20°C, 0.8 mL/min, Vc=0.8x30 cm=15 mL, G2000SW Glass, Cat No 08799, Tosoh Bioscience, 10 µm, without guard column filter.



11. Result: Sample Name: N-24-SE-4-gr-PAMAM. Size Exclusion HPLC. PAMAM, 20% in MeOH, (5 µl, 8.75 $A_{220}U$, in 50 µL of buffer D, monitored at λ=220 nm). Isocratic D/80 min, D=50% CH_3CN, 0.1% CF_3CO_2H in H_2O, T = 20°C, 0.8 mL/min, Vc=0.8x30 cm=15 mL, G2000SW Glass, Cat No 08799, Tosoh Bioscience, 10 µm, without guard column filter.





12. Conclusions. There was no final PAMAM-spacer-PNA-spacer-peptide product with expected molecular mass = 10900 Da

13. After this and other failed attempts, I realized that the spacer-PNA-spacer-peptide with a disulfide bridge cannot be directly attached to PAMAM. For covalent binding of PNA to PAMAM it is necessary to introduce some phosphomonoester or carboxyl groups to spacer-PNA-spacer-peptide to yield HOOC-spacer-PNA-spacer-peptide that could be activated with

some activating or coupling reagents, then make reaction of activated HOOC-spacer-PNA-spacer-peptide with amino groups of PAMAM.

14. This scheme of the covalent binding of HOOC-spacer-PNA-spacer-peptide to PAMAM dendrimer cannot work. Activated carboxyl groups are much more active than activated phosphate groups. During activation with carbodiimides or EDAC they could easily react with aromatic amino groups of HOOC-spacer-PNA-spacer-peptide to give many intermolecular side products.

15. Also the HOOC-spacer-PNA-spacer-peptide can easily precipitate when some buffer components are added to the reaction mixture in water. HOOC-spacer-PNA-spacer-peptide is highly soluble only in acidic conditions in the presence of 0.1% CF_3CO_2H. PNA displays extremely low solubility at pH 7, 9 or 12. Furthermore, activated carboxylates on our HOOC-spacer-PNA-spacer-peptide could and would react with amino groups of the Lysine residue in the D(Cys-Ser-Lys-Cys) disulfide-bridged peptide, which would deactivate the activated HOOC-spacer-PNA-spacer-peptide. Such a HOOC-spacer-PNA-spacer-peptide crosslinked to numerous other HOOC-spacer-PNA-spacer-peptide molecules via base aromatic amines and Lysine sidechain amines cannot react with and form a covalent bond with PAMAM dendrimers.

16. To avoid these circumstances, HOOC-spacer-PNA-spacer-peptide should be completely protected, disulfide-bridged, and soluble in compatible organic solvents, which is possible on the solid phase before cleavage and deprotection procedures. However, PAMAM is a huge molecule and could not be attached to HOOC-spacer-PNA-spacer-peptide on solid phase because of steric problems. So, there are no apparent ways to attach HOOC-spacer-PNA-spacer-peptide to PAMAM.

17. After I realized the uselessness of the PAMAM approach, it was necessary to completely change the strategy of the synthesis of dendrimer-spacer-PNA-spacer-peptides. One example of an alternative method of synthesis was that under my direction, members of my laboratory synthesized the peptide on a polymer support, then extended the spacer-PNA-spacer from the N-terminus of the peptide, then extended the polydiamidopropanoate dendrimers from the N-terminus of the last spacer. That is to say, one example of a possible alternative method of

synthesis we developed was to avoid the use of prepared dendrimer molecules, but instead we created our own dendrimers on the end of the spacer-PNA-spacer-peptide as part of a continuous solid phase synthesis.

18. Therefore, attempting to use the teachings of Tomalia to reach the claimed invention was unsuccessful, thereby showing that it would require a substantial reconstruction and redesign of the elements shown in the primary reference as well as a change in the basic principle under which the primary reference construction was designed to operate. Furthermore, following the cited reference, one of ordinary skill in the art would have lacked motivation to use this reference to make the composition of the present invention with a reasonable expectation of success because such motivation to modify the reference(s) is not present. Absent such reasonable motivation, the present invention would not have been obvious to a person of ordinary skill in the art in light of the cited references.

19. Accordingly, since Tomalia et al. (US Patent No. 5,714,166), in view of both Meade et al. (US Patent No. 6,713,046) and Basu et al. (Bioconjugate Chemistry 1997, 8, 481-466) does not teach or suggest every element of claims 1, 3, 4, 7-14, 16, 26-31, 34, 41-45, 48, 50, 52, 54-56, 69-73, 75, 80, 83, 85, 86, and 88-92 the claims are not obvious.

20. I understand from my review of the Office Action that claims 1, 3, 4, 7-14, 16, 26-34, 41-45, 48, 49-52, 54-56, 69-73, 75, 80, 82, 83, 86, and 88-92 stand rejected under 35 U.S.C. §103(a) as being obvious over Tomalia et al., taken with both Meade et al. and Basu et al., in further view of Nakano et al.

21. According to the attorneys, finding obviousness requires that the prior art reference (or references when combined) must teach or suggest all the claim limitations and that there must be some suggestion of motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings.

22. I am very familiar with the Tomalia, Meade, Basu, and Nakano references.

23. As set forth above, attempting to use the teachings of Tomalia to reach the claimed invention was unsuccessful, thereby showing that it would require a substantial reconstruction and redesign of the elements shown in the primary reference as well as a change

in the basic principle under which the primary reference construction was designed to operate. Further, following the cited reference, one of ordinary skill in the art would have lacked motivation to use this reference to make the composition of the present invention with a reasonable expectation of success because such motivation to modify the reference(s) is not present. Absent such reasonable motivation, the present invention would not have been obvious to a person of ordinary skill in the art in light of the cited references.

24. None of the cited references (alone or in combination) describes a compound X-L1-P-L2-T, wherein X represents a diagnostic or therapeutic agent, such as a radionuclide chelated to a dendrimer, P represents a PNA that can bind a nucleic acid, and T represents a cell surface target director, such as a moiety that can bind a cell-surface molecule, and wherein X, P and T are associated with identical or different spacers L1 and L2 to prevent steric hindrance, and therefore lack teaching or suggesting all the claim limitations, for reasons set forth above.

25. Further, following the cited references, one of ordinary skill in the art would have lacked motivation to use this reference to make the composition of the present invention with a reasonable expectation of success because such motivation to modify the reference(s) is not present. Absent such reasonable motivation, the present invention would not have been obvious to a person of ordinary skill in the art in light of the cited references.

26. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: 16 Dec '07

Eric Wickstrom

Eric Wickstrom, Ph.D.